REMARKS

Claims 1 and 7 have been cancelled. New claims 8-11 have been added. As such, claims 2-6 and 8-11 are pending. Support for new claims 8 and 9 may be found on pages 20-24 of the specification. Support for new claims 10 and 11 may be found on page 9 of the specification. No new matter has been added with to the specification.

Claim to priority of JP 9-252541

The Examiner asserts that Applicants have not perfected their claim to priority under 35 U.S.C. §119 to Japanese application No. 9-252541 because a certified copy of the Japanese application has not been filed.

The present application has been filed as a national stage application of International App. No. PCT/JP98/04187 under 35 U.S.C. §371. The Notice of Acceptance of Application under 35 U.S.C. §371 issued on April 28, 2000, a copy of which is attached hereto, indicates that a certified copy of the priority document has been forwarded to the U.S.P.T.O. by the International Bureau and that the U.S.P.T.O. acknowledges receipt of the document. As such, Applicants do not need to separately submit a certified copy of the priority document. Acknowledgement of the claim to priority is therefore respectfully requested.

Objections to the specification

The specification has been objected to for containing typographical errors. The specification has been reviewed and amended, as indicated above, to correct typographical errors. Withdrawal of the objections is respectfully requested.

Objections to the claims

Claim 2 has been objected to as being unclear as to whether Applicants are referring to just one amino acid or both amino acids being deleted or substituted. Claim 2 has been amended to more clearly indicate that both the 129th and 130th amino acids are deleted or substituted and at least one amino acid residue from the 111th to the 128th amino acids or at least one amino acid from the 131st to 133rd amino acids are deleted or substituted.

Claim 3 has been objected to as being unclear as to whether just one or all sixty-one amino acids are deleted or substituted. Claim 3 has been similarly amended to more clearly indicate that all of the amino acids from the 8th to 69th residues have been deleted, amino acid residues 129 and 130 are both either deleted or substituted and at least one amino acid residue from the 111th to the 128th amino acids or at least one amino acid from the 131st to 133rd amino acids are deleted or substituted.

Withdrawal of the objections is therefore respectfully requested.

Rejection of the claims under 35 U.S.C. §112, first paragraph

Claim 1 has been rejected under 35 U.S.C. §112, first paragraph with the assertion that the specification does not describe the claimed subject matter in such a way so as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed, had possession of the claimed invention.

The Examiner asserts that claim 1 reads on all possible Fas ligand derivatives that are resistant to any and all proteases. The Examiner raises the following specific issues.

a) The Examiner asserts that Applicants were only in possession of the Fas ligand derivatives of SEQ ID NOS:1 and 2 and that the claims encompass many structurally unrelated amino acids.

b) The Examiner further asserts that Applicants have not identified the protease to which the FasL is resistant and that the claims encompass a vast number of FasL derivatives that can be produced from a number of proteases.

Claim 1 has been cancelled, thus rendering this rejection moot.

Claim 7 has been rejected under 35 U.S.C. §112, first paragraph as lacking enablement. More specifically, the Examiner asserts that the specification does not teach how to treat any disease involving apoptosis in any animal. The Examiner further asserts that the specification has only been enabled for the in vitro treatment of hepatocytes with FasL and that the field of

the invention has a level of unpredictability such that in vivo data is required. In support of the level of unpredictability of the field, the Examiner relies on Hurtenbach et al. (1993), wherein it is stated that "peptides are currently unsuitable for human therapeutic use." The Examiner also states that the invention is unpredictable in the absence of in vivo data because the 1) the peptide may be inactivated prior to producing an effect; 2) the peptide may not reach its target and 3) the peptide may have other properties that make it unsuitable for in vivo therapeutics.

Claim 7 has been cancelled, thus rendering this rejection moot.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-5 and 7 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite in the recitation of "derivative." Claims 1-5 and 7 have been amended for clarity to recite a "polypeptide."

Claim 6 has been rejected as being indefinite in the recitation of "apoptosis regulator." Claim 6 has been amended to more clearly recite a soluble Fas ligand which inhibits Fas-mediated apoptosis, thus more clearly defining the apoptosis regulation by the invention of claim 6. Support for the amendment to claim 6 may be found on page 13, line 1 though page 14, line 20 and page 41, line 5 through page 42, line 8 of the specification.

Claim 7 has been rejected as being indefinite in the recitation of "Fas ligand-induced apoptosis is involved." Claim 7 has been cancelled, thus rendering this rejection moot.

Rejections under 35 U.S.C.§102

Claims 1 and 5-7 have been rejected under 35 U.S.C. §102(a) as being anticipated by WO 98/21232. The Abstract and page 3, lines 6-16 of WO '232 are asserted to disclose a non-cleavable FasL derivative of SEQ ID NO:12, which is considered to be an apoptosis regulator.

The embodiment of the present invention as encompassed by claim 2 is drawn to a FasL polypeptide having the amino acid sequence of natural FasL with the following modifications:

1) amino acids 129 and 130, as measured from the N-terminus, are both either deleted or substituted; and

2) a) at least one amino acid from amino acid residues 111 to 128, or

b) at least one amino acid from amino acid residues 131 to 133 are deleted or substituted.

The invention as encompassed by claim 3 has the additional feature that amino acids 8-69, as measured from the N-terminus, are deleted.

The invention of claims 4 and 5 further define this first embodiment of the invention as being a polypeptide of SEQ ID NO:1 or 2 or the DNA encoding the sequences.

The embodiment of the invention encompassed by claims 2-5 is drawn to a non-soluble, i.e. membrane binding, form of FasL that has reduced protease sensitivity and that because of the properties of reduced protease sensitivity and non-solubility has apoptotic cytotoxicity.

The second embodiment of the invention is encompassed by claim 6 and is drawn to a soluble FasL. As recited in claim 6 the soluble FasL polypeptide inhibits Fas-mediated apoptosis.

Claims 1 and 7 have been cancelled, thus the rejection of claims 5 and 6 as being anticipated by WO '232 remains, however is overcome by the following amendments and remarks.

Claim 5 has been amended to depend from claim 2, which has not been rejected over WO '232. As such, the rejection of claim 5 is overcome.

Claim 6 has been amended to be drawn to a soluble Fas ligand which inhibits Fas-mediated apoptosis. WO '232 discloses some deletion mutants of Fas ligand. However, WO '232 does not disclose soluble forms of Fas ligand or soluble forms of Fas ligand, which inhibit Fas-mediated apoptosis. As such, the invention of claim 6 is not anticipated by WO '232 and withdrawal of the rejection is respectfully requested.

Claims 2, 3, 5 and 6 have been rejected under 35 U.S.C. §102(b) as being anticipated by Suda et al. Suda et al. is asserted to disclose on page 1171, Figure 2, a FasL derivative having the 129th amino acid from the N-terminus and at least one

of the 111th to 128th and 131st to 133rd amino acids substituted. The Examiner relies on the disclosure of Accession No. 49266 as evidence that amino acid residue 129 is substituted and that at least one of amino acid residues 111-128 and 131-133 are substituted in Suda et al.

As noted above, the present invention as encompassed by claim 3 requires the deletion of amino acid residues 8-69, as measured from the N-terminus. Suda et al. is silent about the deletion of any amino acids. As such, the invention of claim 3 is not anticipated by Suda et al. and withdrawal of the rejection of claim 3 is respectfully requested.

The invention of claim 2 requires that amino acid residue 129 of natural human Fas ligand is substituted or deleted. Contrary to the assertion of the Examiner, Suda et al. does not disclose the substitution of amino acid 129, as measured from the N-terminal end. Suda et al. disclose, in Figure 2, the sequence for natural rat Fas Ligand. Attached hereto is the alignment of the rat Fas ligand of Accession No. A49266 of Suda et al. and natural human Fas ligand. The alignment clearly shows that amino acid residue 129, as measured from the amino terminus, is lysine in both sequences. Thus, Suda et al. does not disclose the feature of claim 2, of the 129th amino acid residue of the natural human Fas ligand being substituted. As such, the present invention, as encompassed by claim 2 is not anticipated by Suda et al. and withdrawal of the rejection is respectfully requested.

Claim 6 has been further rejected over Suda et al. with the assertion by the Examiner that the Fas ligand proteins of Suda et al. are intrinsically an apoptosis regulator, including a soluble Fas ligand. As noted above, Figure 2 of Suda et al. discloses the natural rat Fas ligand. Figure 4 of page 1172 discloses that the Fas ligand protein of Suda et al. has cytotoxic activity. The invention as embodied by claim 6, on the other hand, is specifically drawn to soluble Fas ligand that inhibits Fas-mediated apoptosis (cytotoxicity), i.e. the opposite of the activity of Suda et al. As such, the invention of claim 6 is distinguished from disclosure of Suda et al. and withdrawal of the rejection of claim 6 is respectfully requested.

As the above-indicated amendments and remarks address and overcome the objections and rejections of the specification and claims, withdrawal of the objections and rejections and allowance of the claims are respectfully requested.

Should the Examiner have any questions regarding the above-indicated application she is requested to please contact MaryAnne Armstrong, PhD (Reg. No. 40,069) in the Washington DC area at (703) 205-8000.

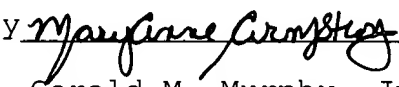
A marked-up version of the specification and claims showing amendments is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional

fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 

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GMM/MAA
1110-266P

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

The paragraph beginning on page 5, line 13, has been amended as follows:

Fas ligand is a type II membrane protein which belongs to the tumor necrosis factor (TNF) family, and induces apoptosis by binding to Fas which is the receptor. FasL is cleaved by a putative processing enzyme, metalloproteinase to produce a soluble form. The inventors of the present invention purified human soluble FasL from the supernatant of the transformant mouse cell expressing human FasL, and identified the cleavage site. Deletion of 4 to 23 amino acids around the cleavage site blocked shedding of the human FasL from the membrane while the apoptosis-inducing activity was retained. Mouse WR19L ~~cell~~ cells overexpressing the Fas is ~~sentitive~~ sensitive to membrane-bound FasL as in the case with the FasL of soluble form whereas Jurkat cells and mouse primary hepatocytes which endogenously express a low level of Fas exhibited resistance to soluble FasL. When the membrane-bound FasL was used as an ~~effecter~~ effector, the human Jurkat cells and the mouse hepatocytes were efficiently killed. Furthermore, soluble FasL inhibited cytotoxicity of the membrane-bound FasL to the mouse hepatocyte. These results indicate that the membrane-bound form of FasL is the functional form, and its activity is

downregulated by the shedding of the soluble FasL from the membrane.

The paragraph beginning on page 7, line 16, has been amended as follows:

FIG. 2 is a schematic ~~diagrams~~ diagram of the FasL constructs carrying deletion or point mutation.

The paragraph beginning on page 11, line 14, has been amended as follows:

It should be noted that the amino acid sequences shown in SEQ ID No. 1 and 2 have four ~~glycosilation~~ glycosylation sites (~~N-glycosilation~~ N-glycosylation sites), respectively. In SEQ ID No. 1, amino acid numbers 76 - 78, 161 - 163, 227 - 229, and 237 - 239, and in SEQ ID No. 2, amino acid numbers 76 - 78, 180 - 182, 246 - 248, and 256 - 258 correspond to such ~~glycosilation~~ glycosylation sites. The novel FasL derivative of the present invention may also have a sugar chain added thereto at such site.

The paragraph beginning on page 13, line 12, has been amended as follows:

The soluble Fas ligand of the present invention is the ligand which shares at least some region with the natural Fas ligand; which is soluble in an aqueous solution in the absence of a

surfactant; and which interacts with the extracellular region of the Fas to compete with the natural FasL or to induce downregulation of the Fas. Exemplary such soluble Fas ligand is the one comprising at least some of the extracellular region of the Fas ligand, and a preferable example of the soluble Fas ligand is a polypeptide comprising the amino acid sequence of human natural Fas ligand from Gln 130 from N terminal to the C terminal.

The paragraph beginning on page 15, line 9, has been amended as follows:

Exemplary heart diseases include ischemic heart diseases such as myocardial infarction, myocarditis of various causes, cardiomyopathy, in particular, dilated cardiomyopathy, cardiac insufficiency, and ischemic reperfusion injury and diseases caused by such ischemic reperfusion injury. Exemplary GVHD include GVHD after bone marrow transplantation such as incompatible bone marrow transplantation and bone marrow transplantation in the case of congenital immunodeficiency; GVHD after organ transplantation; GVHD after blood infusion of large amount to the host with reduced immunocompetence. Ischemic reperfusion injuries include ischemic reperfusion injuries found in liver, heart, kidney, lung, spleen, small intestine, large intestine, ~~stomac~~ stomach, pancreas, brain, muscle, skin, and the like as well as diseases caused by such ischemic reperfusion injury such as hepatic insufficiency, reperfusion arrhythmia, renal insufficiency, necrotizing

enterocolitis, and other injuries and dysfunction of various organs.

In the Claims:

Claims 1 and 7 have been canceled.

The claims have been amended as follows:

2. A novel ~~Fas ligand derivative~~ polypeptide having an amino acid sequence of natural human Fas ligand wherein the 129th amino acid - and 130th amino acid residues as measured from N terminal end are both deleted or substituted, and at least one amino acid residue of from 111th amino acid - to 128th amino acid residues or at least one amino acid residue of from 131st amino acid - to 133rd amino acid residues as measured from N terminal end is deleted or substituted.

3. A novel ~~Fas ligand derivative~~ polypeptide having an amino acid sequence of natural human Fas ligand wherein all of the 8th amino acid - to 69th amino acid residues as measured from N terminal end are deleted, 129th amino acid - and 130th amino acid residues as measured from N terminal end are both deleted or substituted, and at least one amino acid residue of from 111th amino acid - to 128th amino acid residues or at least one amino acid residues from 131st amino acid - to 133rd amino acid residues as measured from N terminal end is deleted or substituted.

4. A novel ~~Fas ligand derivative~~ polypeptide including the amino acid sequence described in SEQ ID NO. 1 or 2.

5. A DNA coding for the novel ~~Fas ligand derivative~~ polypeptide of ~~any one of claims 1 to 4~~ claim 2.

6. ~~An apoptosis regulator including a~~ A soluble Fas ligand which inhibits Fas-mediated apoptosis.

Claims 8-11 have been added.

CLUSTAL W (1.74) multiple sequence alignment

```
RatFasL_Accession49266_     MQQPVNYPCPQIYWVDSSATSPWAPPGSVFSCPSSGPRGPGQRRPPPPPP
natural_human_FasL          MQQPFNYPYPQIYWVDSSASSPWAPPGTVLPCPTSVPRRPGQRRPPPPPP
                            ****.*** **********:*******:*:.**:* ** **********

RatFasL_Accession49266_     PPSPLPPPSQPPPLPPLS--PLKKK--DNIELWLPVIFFMVLVALVGMGL
natural_human_FasL          PP-PLPPPPPPPLPPLPLPPLKKRGNHSTGLCLLVMFFMVLVALVGLGL
                            ** *****. *******.  ****:  ..  * * *:**********:**

                                                 129
RatFasL_Accession49266_     GMYQLFHLQKELAELREFTNHSLRVSSFEKQIANPSTPSETKKPRSVAHL
natural_human_FasL          GMFQLFHLQKELAELRESTSQMHTASSLEKQIGHPSPPPEKKELRKVAHL
                            **:****************.*.: . .**:****.:**.*.*.*: *.****

RatFasL_Accession49266_     TGNPRSRSIPLEWEDTYGTALISGVKYKKGGLVINEAGLYFVYSKVYFRG
natural_human_FasL          TGKSNSRSMPLEWEDTYGIVLLSGVKYKKGGLVINETGLYFVYSKVYFRG
                            **:..***:********* .*:**************:**************

RatFasL_Accession49266_     QSCNSQPLSHKVYMRNFKYPGDLVLMEEKKLNYCTTGQIWAHSSYLGAVF
natural_human_FasL          QSCNNLPLSHKVYMRNSKYPQDLVMMEGKMMSYCTTGQMWARSSYLGAVF
                            ****. ********** *** ***:** * :.******:**:********

RatFasL_Accession49266_     NLTVADHLYVNISQLSLINFEESKTFFGLYKL
natural_human_FasL          NLTSADHLYVNVSELSLVNFEESQTFFGLYKL
                            *** *******:*:***:*****:********
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OIPE JC152 AUG 22 2001 PATENT & TRADEMARK OFFICE DEPARTMENT OF COMMERCE UNITED STATES OF AMERICA

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

RECEIVED AUG 28 2001 TECH CENTER 1600/2900

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09/508849	NAGATA S	1110-266PCT

INTERNATIONAL APPLICATION NO.
PCT/JP98/04187

I.A. FILING DATE	PRIORITY DATE.
17 SEP 98	17 SEP 97

BIRCH STEWART KOLASCH & BIRCH
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FALLS CHURCH, VA 22040 0747

DATE MAILED: 28 APR 2000

NOTIFICATION OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

1. The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as ☐ a Designated Office (37 CFR 1.494), ☒ an Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is **ACCEPTED** for national patentability examination in the United States Patent and Trademark Office.

2. The United States Application Number assigned to the application is shown above and the relevant dates are:

17 MAR 2000 — 35 U.S.C. 102(e) DATE

17 MAR 2000 — DATE OF RECEIPT OF 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE "FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371(C) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** *The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363).* Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

3. ☒ A request for immediate examination under 35 U.S.C. 371(f) was received on 17 MAR 2000 and the application will be examined in turn.

4. The following items have been received:
- ☒ U.S. Basic National Fee.
- ☒ Copy of the international application in:
  - ☒ a non-English language.
  - ☐ English.
- ☒ Translation of the international application into English.
- ☒ Oath or Declaration of inventors(s) for DO/EO/US.
- ☐ Copy of Article 19 amendments. ☐ Translation of Article 19 amendments into English.
  The Article 19 amendments ☐ have ☐ have not been entered.
- ☒ The International Preliminary Examination Report in English and its Annexes, if any.
- ☐ Copy of the Annexes to the International Preliminary Examination Report (IPER).
  - ☐ Translation of Annexes to the IPER into English.
  - The Annexes ☐ have ☐ have not been entered.
- ☒ Preliminary amendment(s) filed 17 MAR 2000 and _____.
- ☒ Information Disclosure Statement(s) filed 17 MAR 2000 and _____.
- ☒ Assignment document.
- ☐ Power of Attorney and/or Change of Address.
- ☐ Substitute specification filed _____.
- ☐ Verified Statement Claiming Small Entity Status.
- ☒ Priority Document.
- ☒ Copy of the International Search Report ☒ and copies of the references cited therein.
- ☐ Other:

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above. (37 CFR 1.5)

Fred Smith
Telephone: 703-305-3654

FORM PCT/DO/EO/903 (December 1997)